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Thymosin alpha 1 in the prevention of pancreatic infection following acute necrotizing pancreatitis (TRACE trial): protocol of a multicenter, randomized, double-blind, placebo-controlled, parallel-group, trial

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Thymosin alpha 1 in the prevention of pancreatic infection following acute necrotizing pancreatitis (TRACE trial): protocol of a multicenter, randomized, double-blind, placebo-controlled, parallel-group, trial

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Abstract

Introduction: Infected pancreatic necrosis (IPN) and its related septic complications are the major causes of death in patients with acute necrotizing pancreatitis (ANP). Therefore, the prevention of pancreatic infection is of great clinical value for the treatment of ANP. This study was designed to evaluate the efficacy and safety of Thymosin Alpha 1 among patients with ANP.

Methods/Design: This is a prospective, randomized, multicenter, double-blind, placebo-controlled study. 520 eligible ANP patients will be randomized in a 1:1 ratio to receive either the Thymosin alpha 1 or the placebo using the same mode of administration. The primary endpoint is the incidence of IPN during the index admission. Most of the secondary endpoints will be registered through the index admission including in-hospital mortality, incidence of new-onset organ failure and new-onset persistent organ failure (respiration, cardiovascular and renal), receipt of new organ support therapy, requirement for drainage or necrosectomy, bleeding requiring intervention, HLA-DR on day0, day7 and day14, etc. and adverse events. Considering the possibility of readmission, an additional follow-up will be arranged 90 days after enrollment, and IPN and death at Day90 will also be served as secondary outcomes.

Ethics and dissemination: This study was approved by the ethics committee of Jinling Hospital, Nanjing University (No. 2015NZKY-004-02). The TRACE trial was designed to test the effect of a new therapy focusing on the immune system in preventing secondary infection following ANP. The results of this trial will be disseminated in peer-reviewed journals and at scientific conferences.

Trial registration： The trial has been registered at the ClinicalTrials.gov registry (NCT02473406)

Strengths and limitations of this study

Strength 1：This is a randomized, multicenter, double-blind, placebo-controlled trial providing top-class evidence concerning the efficacy and safety of thymosin alpha 1 for patients with ANP.

Strength 2: Based on the favorable safety profile of thymosin alpha 1, no serious

drug-related adverse event was expected and the data will be handled by an independent data safety monitoring board (DSMB).

Limitation 1: As infection is a relatively low-incidence complication of ANP, a sample size of 520 will be required to detect the efficacy of Thymosin Alpha 1, which will take years before the final conclusion could be drawn.

Limitation 2: Due to the limited resources we could utilize, continuous immune function assessment is impossible in this study. Alternatively, we opted to appraise the immune status with predesigned time points.

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Background

Infected pancreatic necrosis (IPN) and its related septic complications contribute substantially to deaths in patients with acute necrotizing pancreatitis(ANP)[1]. Compared with patients with sterile necrosis, those with IPN suffered a significant increase in mortality ranging from 14% to 69%, despite advances in critical care, surgical and endoscopic interventions and antibiotics[2]. Therefore, the prevention of pancreatic necrosis infection is of great clinical value in the treatment of ANP. Over the past years, numerous attempts had been made to prevent or delay the development of IPN including antibiotic prophylaxis, early enteral nutritional, selective gut decontamination and probiotics, but none of them had been proved to improve patient-centered outcomes with high-quality evidence [3-6]. More efficacious treatment aiming at reducing infectious complications of ANP is in need.

Immunosuppression and disorders characterized by decreased HLA-DR expression and unbalanced CD3/CD4+/CD8+ T cells of peripheral blood mononuclear cell (PBMC) are reported to be associated with IPN[7, 8], especially in those with a more severe type of disease, whose suppressed immune function occurs early and strongly[8, 9]. Our previous observational study found that early enteral nutrition could moderate the excessive immune response during the early stage of severe acute pancreatitis without leading to subsequent immunosuppression, and ultimately reduce the incidence of infection and ICU stay [10].Thus, immunomodulatory treatment could potentially intervene in the evolution of secondary infection of pancreatic necrosis, resulting in better outcomes. Efforts had been made in this field using drugs like lexipafant and octreotide, but the hitherto existing evidence failed to show solid clinical benefits of immunomodulation with regard to major clinical outcomes [11].

Thymosin alpha 1 had been shown to have immunomodulatory properties and was reported to be clinically beneficial in patients with sepsis[12, 13], majorly through the involvement of distinct Toll-like receptors (TLRs) acting on different dendritic cells (DC) subsets and involving the MyD88-dependent signaling pathway. However, for acute pancreatitis, the only randomized controlled study was the pilot one conducted by our group years ago, suggesting that the use of Thymosin alpha 1 was associated

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with improved cellular immunity and reduced infection rate in a group of 24 patients[14]. Due to the nature of the small sample size and single-center, the clinical implication and generalizability of this study are thought to be limited. Therefore, thymosin alpha 1 in the prevention of pancreatic infection following acute necrotizing pancreatitis (TRACE trial) was designed with sufficient power performed in 16 hospitals in China

Study objectives

The primary objective of the TRACE trial is to determine whether Thymosin Alpha 1 is superior to placebo in reducing the incidence of infected pancreatic necrosis in patients with acute necrotic pancreatitis.Secondary objectives are to determine the safety and the impact on immune function from Thymosin Alpha 1 among patients with ANP.

Study Design

The present study (the TRACE trial) is an investigator-initiated, multicenter, individually-randomized, double-blind, placebo-controlled, parallel-group study. This trial was registered on June 16, 2015, in the CT.gov registry (NCT02473406, https://www.clinicaltrials.gov/) and was approved by the ethics committee of Jinling Hospital, Nanjing University (No. 2015NZKY-004-02). Local ethics approval was also obtained before enrollment in each participating center. The TRACE trial was designed and coordinated by the Center of Severe Acute Pancreatitis at Nanjing University and the coordinating and data management center of the Chinese Acute Pancreatitis Clinical Trials Group (CAPCTG). The trial steering committee (TSC) was formed to oversee the implementation of the study, and a data safety monitoring board (DSMB) will regularly (every 6 months) review the safety report prepared by the trial statistician from the accumulating data of this trial.

Study population

This clinical trial is performed in 16 hospitals from China. All adult patients with AP admitted to the participating centers will be assessed for eligibility after

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admission. The inclusion and exclusion criteria are as follows:

Inclusion criteria

1. Symptoms and signs of acute pancreatitis based on abdominal pain suggestive of AP, serum amylase at least three times the upper limit of normal, and/or characteristic findings of AP on computed tomography or less commonly magnetic resonance imaging (MRI) or transabdominal ultrasonography according to the Revised Atlanta Criteria[15];

2.Less than one week from the onset of abdominal pain;

3. Age between 18 to 70 years old;

4. Acute Physiology and Chronic Health Evaluation(APACHE II) score ≥8 during the last 24 hours before enrollment

5.Balthazar CT score ≥5 (presence of pancreatic necrosis)[16].

6. Written informed consent obtained

Exclusion criteria

1. Pregnant pancreatitis;
2. History of chronic pancreatitis；
3. Malignancy related acute pancreatitis
4. Receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before admission；
5. Patients with a known history of severe cardiovascular, respiratory, renal or hepatic diseases defined as (1) greater than New York Heart Association Class II heart failure(Class II not included), (2) active myocardial ischemia or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with requirement for home oxygen;
6. Patients with preexisting immune disorders such as AIDS.

A patient will be considered eligible if he/she meets the inclusion criteria and does not meet any of the exclusion criteria. Allocation will be performed after signed consent is obtained. The study protocol flow of participants is outlined in Figure 1.

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Randomization and blinding methods

After the completion of screening measurements and the acquisition of written informed consent, eligible participants will be randomized in a 1:1 ratio to either the treatment group or the placebo group. The randomization code was computer-generated with a block size of 4.

Participants, clinical investigators, and investigators assessing outcome data will be blinded to the treatment allocation to minimize potential sources of bias. The trial statistician will also be blinded regarding the treatment code when developing the statistical programs, which will be validated and completed using dummy randomization codes. The actual allocation will only be provided to the study team after locking of the database and approval of the statistical analysis plan.

Trial drugs

After randomization, the participant will receive:

1. Thymosin Alpha 1 1.6mg I.H q12h for the first 7 days and 1.6mg I.H, qd for the following 7 days. The administration will be terminated any day during the treatment when the patient deemed as qualified for discharge, or
2. Matching placebo(normal saline) using the same mode of administration as the above mentioned.

As shown in Figure 1, the recruited patients will start randomized drugs subcutaneously from the day after the allocation day. Thymosin Alpha will be provided by SciClone Pharmaceuticals and the matching placebo by Chengdu Tongde Pharmaceuticals. All study drugs will be stored in a secure area with access limited to the investigators and authorized study site personnel, and under appropriate storage conditions.

General treatment regimen

All patients will receive initial standard treatment including fluid therapy, early enteral nutrition, routine medical treatment like proton pump inhibitor as indicated, mechanical ventilation if needed, and continuous renal replacement therapy (CRRT) if

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needed in the light of recently published guidelines[17]. All participating centers are able to offer appropriate intensive care in case the patients require organ support or continuous monitoring. The necrotic collection will be intervened when infection is suspected or confirmed, but the intervention should be optimally delayed for 4 weeks when the patient could tolerate the symptoms as suggested by the guidelines[17].

When pancreatic infection occurs, either a surgical or endoscopic step-up approach considering the location of the necrotic collection and the technical availability in each participating center will be applied. Initial drainage is optimally performed after the necrotic collection is well walled-off based on guideline recommendations[17]. Principally, either percutaneous catheter drainage or endoscopic transluminal placement of double pig-tail stents，rather than debridement, are the primary choices of treatment.

Endpoints

Primary outcome measure

The incidence of IPN during the index admission will be served as the primary outcome measure of the TRACE trial. The diagnosis of IPN will be based on the international guidelines when one or more of the following were present: gas bubbles within (peri) pancreatic necrosis on computed tomography; a positive culture of (peri) pancreatic necrosis obtained by image-guided fine-needle aspiration; a positive culture of (peri) pancreatic necrosis obtained during the first drainage and/or necrosectomy[15].

Secondary outcome measures

Part I：Secondary outcomes during the index admission

1. The occurrence of new-onset organ failure and new-onset persistent organ failure (SOFA score for respiration, cardiovascular, or renal system ≥2). New-onset is defined as events that occur after randomization and not present 24 hours before randomization;
2. In-hospital mortality;
3. Bleeding requiring intervention；
4. Gastrointestinal perforation or fistula requiring intervention;

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5. Incidence of pancreatic fistula
6. New receipt of mechanical ventilation (not applied 24 hours before randomization);
7. New receipt of renal replacement therapy (not applied 24 hours before randomization);
8. New receipt of vasoactive agents (not applied 24 hours before randomization);
9. The requirement for catheter drainage (either percutaneous or endoscopic)
10. Number of drainage procedures required;
11. The requirement for minimally-invasive debridement;
12. Number of minimally invasive necrosectomy required;
13. The requirement for open surgery;
14. Number of open surgery required;
15. Length of intensive care unit(ICU) stay;
16. Length of hospital stay;
17. SOFA score on day0, day7, and day14；
18. CRP level on day0, day7, and day14;
19. HLA-DR level on day0, day7, and day14;
20. Lymphocyte count on day0, day7 and day 14;
21. In-hospital cost.

Part II：Secondary outcomes within 90 days after enrollment

1. Incidence of infection within 90 days after enrollment；
2. Mortality within 90 days after enrollment;

Sample size estimation

The incidence of pancreatic infection during the index admission was reported to be around 25% in ANP episodes combined with an APACHE II score≥8 in our previous studies[18, 19]. To demonstrate a 40% reduction in the incidence of pancreatic infection on the basis of our pilot study [14], we projected a sample size of 500 participants with 80% power at a two-sided alpha level of 0.05 using the PASS software (PASS 11, NCSS software, Kaysville, USA). In our study , we planned to randomize 520 patients

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after considering 4% of lost follow up.

Statistical analysis

Primary analyses will be based on the intention-to-treat (ITT) population, and secondary supportive analyses will be done on the PP population. The safety analysis will be performed on the safety population. Missing data will be handled by multiple imputations to evaluate the robustness of the primary endpoint analyses[20].The populations are defined as follows:

1. ITT population: This population consists of all randomized subjects, regardless of whether they are ineligible, prematurely discontinue treatment, or are otherwise protocol violators/deviators.
2. Per-protocol (PP) population: This population is a subset of the ITT population. Subjects with major protocol deviations will be excluded from the PP population. Major protocol deviations will be defined in the statistical analysis plan.
3. Safety population: This population will be the same as the ITT population, which consists of all randomized subjects, who receive at least one dose of study drug.

The normality of continuous variables was examined using skewness and kurtosis. Categorical data were expressed as number and percentage. A generalized linear model (GLM) will be employed to compare group differences. No interim unblinding and analysis. Statistical tests will be two-sided, and p values < 0.05 will be accepted as significant. A full definition and explanation of all primary, secondary and pre-defined subgroup analyses such as patients with different severity of AP(severe and non-severe), patients with different age(dichotomized at 60 years old), patients with different etiologies (biliary and non-biliary) and patients with different extent of pancreatic necrosis(>50% and ≤50%) will be included in the statistical analysis plan.

Adverse events

Adverse events (AEs) are defined in accordance with the National Cancer Institute-Common Terminology Criteria for Adverse Events as any untoward medical occurrence in a patient, or clinical investigation subject administered an investigational

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intervention and which does not necessarily have to have a causal relationship with this treatment.

It is recognized that the patient population (ANP with relatively high APACHE II score) will experience a number of common aberrations in laboratory values, signs and symptoms due to the severity of the underlying disease and the impact of standard therapies. These will not necessarily constitute an adverse event unless they require significant intervention or are considered to be of concern in the investigator's clinical judgment. The DSMB will review the safety report every 6 months.

Recruiting process

The trial was registered on June 16, 2015, in the CT.gov registry(NCT02473406 https://www.clinicaltrials.gov). The first patient was randomized on the 22nd of March 2017. So far, 426 patients had been randomized and the enrollment keeps to the schedule.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Data collection and management

A web-based electrical database (access through the website of the CAPCTG, https://capctg.medbit.cn/) will be used for data collection and storage. All data will be input by the primary investigator or nominated investigator (less than two for each participating center) approved by the primary investigator, and a double check will be done by the research coordinator. Training for data entry will be performed by the provider of the electrical database and the coordinating and data management center of the CAPCTG. According to the schedule shown in Figure 2, the investigator will collect data during the index admission and at day 90 after enrollment.

Discussion

The TRACE trial was designed to test the effect of a new therapy focusing on the immune system in preventing secondary infection following ANP, which is a

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potentially lethal complication causing substantial morbidity and mortality. We also aimed to prospectively investigate the effect of immunomodulatory treatment with thymosin alpha 1 in patients with different severity of diseases with predefined subgroup analysis. The results of the TRACE trial would potentially provide a novel therapeutic option in the early management of ANP and identify the patient population who may benefit most from immunomodulation.

Immunomodulation is of significant clinical value in critically ill settings and the treatment of sepsis[12]. While, acute pancreatitis, which has a lot in common with sepsis like overwhelmed inflammation and infection related complications, might be another good target for immunomodulatory therapy. In general, previously studied drugs such as lexipafant and octreotide were aimed to control cytokines, which are thought to be the pivotal part in the early inflammatory response of AP, rather than preventing secondary infection[11]. However, like what we learned in sepsis, immunosuppression quickly following the initial inflammatory cascades should be the target of treatment during the course of ANP, as well, especially in those with organ failure[8, 21]. A pilot study published from our group several years ago indicated that the administration of thymosin α1 could improve compromised monocyte HLA-DR expression and reduce infection rate in a small group of patients (n=24) with severe acute pancreatitis defined by the Atlanta Classification. The result of this study is encouraging which drive us to conduct this large multi-center RCT to obtain more reliable clinical evidences[14].

The TRACE trial was sponsored by the CSAP at Jinling Hospital, Nanjing University, which is the national referral center for acute pancreatitis (AP) admitting more than 600 cases of AP annually and coordinated by the CAPCTG coordinating and data management center, which could cover the whole country. The trial will be performed in 16 centers across China and aims to recruit 520 patients. Due to the limitation of the budget and technical availability, we can not perform comprehensive immune test in multiple time points, and alternatively, we choose monocyte HLA-DR, which is a representative parameter of the immune system majorly reflecting the antigen presentation capacity to assess the immunomodulatory effect of thymosin alpha 1 and

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also be widely used in previous studies regarding immune function in different diseases like sepsis[12, 22].

In conclusion, the TRACE trial aims to assess the efficacy of thymosin α1 administered early during the disease course of ANP on the rate of necrosis infection and other major clinical outcomes and thereby offer a novel therapeutic option in the treatment of ANP patients.

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Figure 1：Trial flow chart.

Figure 2. Schedule for participants enrolment, drug administration, and data collection.

Declarations

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Declarations

Authors' contributions：

Study concept and design: L K, Z T

Acquisition of data; analysis and interpretation of data: J Z, G L, B Y, Z Z, W M

Drafting of the manuscript; J Z, L K

Critical revision of the manuscript for important intellectual content: Z Z, W L

Obtained funding; administrative, technical, or material support: Z T, W L

Study supervision: W L, J L

Competing interests

This study was supported by SBE2016750187 of Science and technology project,Jiangsu Province,China. SciClone Pharmaceuticals in part provided the study drug for this investigatorinitiated study, but had no influence on the study design, data analysis or report. The investigators take full responsibility of the integrity and content of this paper.

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Ethics approval and consent to participate:

This study was approved by the ethic committee of Jinling Hospital. The Ethical Approval Document ID is 2015NZKY-004-02.Even central ethical approval has been confirmed, we will not begin recruiting at other centers in the trial until local ethical approval has been obtained. Besides, the consents for this study were obtained from each patient or his next of kin.

Consent for publication

Not Applicable.

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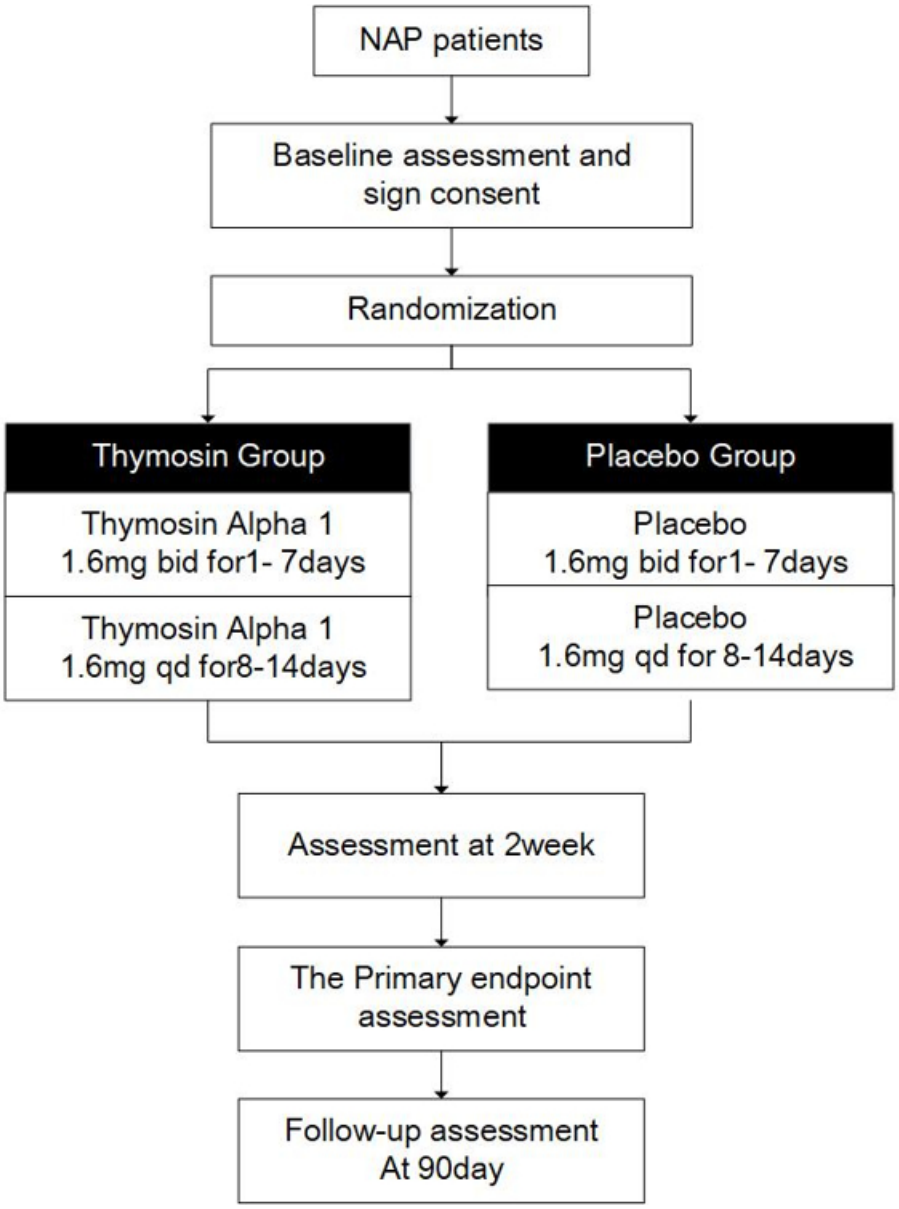

Figure 1 : Trial flow chart.

	Study period							
	Enrollment	Allocation	Index admission					Follow-up
TIMEPOINT	< 24h	0	day1-6	day7	day8-13	day14	discharge/death	90 day
ENROLLMENT								
Eligibility screening	X							
Informed consent	X							
Allocation		X						
INTERVENTIONS:								
Drug injection 1.6mg bid			X	X				
Drug injection 1.6mg qd					X	X		
ASSESSMENTS:								
Incidence of IPN			←	—	—	→		
Major complications			←	—	—	→		
Laboratory test	X			X		X		
Organ failure assessment	X			X		X		
Status of vitality and infection								X

Figure 2. Schedule for participants enrolment, drug administration, and data collection.

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Thymosin alpha 1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis (TRACE trial): protocol of a multicenter, randomized, double-blind, placebo-controlled, parallel-group trial

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Abstract

Introduction: Infected pancreatic necrosis (IPN) and its related septic complications are the major causes of death in patients with acute necrotizing pancreatitis (ANP). Therefore, the prevention of IPN is of great clinical value, and immunomodulatory therapy with Thymosin Alpha 1 may be beneficial. This study was designed to test the hypothesis that the administration of Thymosin Alpha 1 during the acute phase of ANP will result in a reduced incidence of IPN.

Methods and analysis: This is a randomized, multicenter, double-blind, placebo-controlled study. 520 eligible ANP patients will be randomized in a 1:1 ratio to receive either the Thymosin alpha 1 or the placebo using the same mode of administration. The primary endpoint is the incidence of IPN during the index admission. Most of the secondary endpoints will be registered within the index admission including in-hospital mortality, the incidence of new-onset organ failure and new-onset persistent organ failure (respiration, cardiovascular and renal), receipt of new organ support therapy, requirement for drainage or necrosectomy, bleeding requiring intervention, HLA-DR on day0, day7, and day14, etc., and adverse events. Considering the possibility of readmission, an additional follow-up will be arranged 90 days after enrollment, and IPN and death at Day90 will also be served as secondary outcomes.

Ethics and dissemination: This study was approved by the ethics committee of Jinling Hospital, Nanjing University (No. 2015NZKY-004-02). The TRACE trial was designed to test the effect of a new therapy focusing on the immune system in preventing secondary infection following ANP. The results of this trial will be disseminated in peer-reviewed journals and at scientific conferences.

Trial registration： The trial has been registered at the ClinicalTrials.gov registry (NCT02473406)

Strengths and limitations of this study

Strength 1： This is a randomized, multicenter, double-blind, placebo-controlled trial providing top-class evidence concerning the efficacy and safety of thymosin alpha 1 for patients with acute necrotizing pancreatitis.

Strength 2: The data will be handled by an independent data safety monitoring board

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(DSMB) to ensure the safety of the participants.

Strength 3: Thymosin alpha 1 is a well-studied drug with a favorable safety profile in previous trials.

Limitation 1: A sample size of 520 is required to detect the efficacy of Thymosin Alpha 1 in preventing infected pancreatic necrosis, which will take years before the conclusion could be drawn.

Limitation 2: Continuous immune function assessment is not applied in this study.

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Background

Infected pancreatic necrosis (IPN) and its related septic complications contribute substantially to deaths in patients with acute necrotizing pancreatitis(ANP)[1]. Compared with patients with sterile necrosis, those with IPN suffered a significant increase in mortality ranging from 14% to 69%, despite advances in critical care, surgical and endoscopic interventions, and antibiotics[2]. Therefore, the prevention of IPN is of great clinical value in the treatment of ANP. Over the past years, numerous attempts had been made to prevent or delay the development of IPN, including antibiotic prophylaxis, early enteral nutritional, selective gut decontamination, and probiotics. Still, none of them had been proved to improve patient-centered outcomes with high-quality evidence [3-6]. More promising treatment aiming at reducing infectious complications of ANP is in need.

Immunosuppression and disorders characterized by decreased HLA-DR expression and unbalanced CD3/CD4+/CD8+ T cells of peripheral blood mononuclear cell are reported to be associated with IPN[7, 8], especially in those with a more severe type of disease, whose suppressed immune function occurs early and strongly[8, 9]. Our previous observational study found that early enteral nutrition could moderate the excessive immune response during the acute phase of severe acute pancreatitis without leading to subsequent immunosuppression, and ultimately reduce the incidence of infection and ICU stay [10]. Thus, immunomodulatory treatment could potentially intervene in the development of IPN, resulting in better outcomes. Efforts had been made in this field using drugs like lexipafant and octreotide. Still, the hitherto existing evidence failed to show robust clinical benefits of immunomodulation with regard to key clinical outcomes [11].

Thymosin alpha 1 had been shown to have immunomodulatory properties and was reported to be clinically beneficial in patients with sepsis[12, 13], majorly through the involvement of distinct Toll-like receptors acting on different dendritic cells subsets and involving the MyD88-dependent signaling pathway. However, for acute pancreatitis (AP), the only randomized controlled study was the pilot one conducted by our group years ago, suggesting that the use of Thymosin alpha 1 was associated with

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improved cellular immunity and reduced infection rate in a group of 24 patients[14]. Due to the single-center set and small sample size, the clinical implication and generalizability of this study are thought to be limited. Therefore, we designed this multicenter trial, the Thymosin Alpha 1 in the Prevention of Infected Pancreatic Necrosis Following Acute Necrotizing Pancreatitis (TRACE), with sufficient power to test the hypothesis that the administration of Thymosin Alpha 1 during the acute phase of ANP will result in a reduced incidence of IPN.

Study objectives

The primary objective of the TRACE trial is to determine whether Thymosin Alpha 1 is superior to placebo in reducing the incidence of IPN in patients with ANP. Secondary objectives are to determine the safety and the impact on the immune function of Thymosin Alpha 1 among patients with ANP.

Study Design

The present study is an investigator-initiated, multicenter, individually-randomized, double-blind, placebo-controlled, parallel-group study. This trial was registered on June 16th, 2015, in the CT.gov registry (NCT02473406, https://www.clinicaltrials.gov/) and was approved by the ethics committee of Jinling Hospital, Nanjing University (No. 2015NZKY-004-02). Local ethics approval was also obtained before enrollment in each participating center. The TRACE trial was designed and coordinated by the Center of Severe Acute Pancreatitis at Nanjing University and the coordinating and data management center of the Chinese Acute Pancreatitis Clinical Trials Group (CAPCTG). The trial steering committee (TSC) was formed to oversee the implementation of the study, and a data safety monitoring board (DSMB) will regularly (every six months) review the safety report prepared by the trial statistician from the accumulating data of this trial.

Study population

This trial is performed in 16 hospitals from China. All adult patients with AP admitted to the participating centers will be assessed for eligibility after admission. The

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inclusion and exclusion criteria are as follows:

Inclusion criteria

1. Symptoms and signs of AP based on abdominal pain suggestive of AP, serum amylase at least three times the upper limit of normal, and/or characteristic findings of AP on computed tomography or less commonly magnetic resonance imaging (MRI) or transabdominal ultrasonography according to the Revised Atlanta Criteria[15];
2. Less than one week from the onset of abdominal pain;
3. Age between 18 to 70 years old;
4. Acute Physiology and Chronic Health Evaluation(APACHE II) score ≥eight during the last 24 hours before enrollment
5. Balthazar CT score ≥5 (presence of pancreatic necrosis)[16].
6. Written informed consent obtained

Exclusion criteria

1. Pregnant pancreatitis;
2. History of chronic pancreatitis；
3. Malignancy related acute pancreatitis
4. Receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before admission；
5. Patients with a known history of severe cardiovascular, respiratory, renal or hepatic diseases defined as (1) greater than New York Heart Association Class II heart failure(Class II not included), (2) active myocardial ischemia or (3) cardiovascular intervention within previous 60 days, (4) history of cirrhosis or (5) chronic kidney disease with creatinine clearance< 40 mL/min, or (6) chronic obstructive pulmonary disease with the requirement for home oxygen;
6. Patients with preexisting immune disorders such as AIDS.

A patient will be considered eligible if he/she meets the inclusion criteria and does not meet any of the exclusion criteria. Allocation will be performed after signed consent is obtained. The study protocol flow of participants is outlined in Figure 1.

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Randomization and blinding methods

After the completion of screening measurements and the acquisition of written informed consent, eligible participants will be randomized in a 1:1 ratio to either the treatment group or the placebo group. The randomization code was computer-generated with a block size of 4, and the randomization was stratified by sites.

Participants, clinical investigators, and investigators assessing outcome data will be blinded to the treatment allocation to minimize potential sources of bias. The trial statistician will also be blinded regarding the treatment code when developing the statistical programs, which will be validated and completed using dummy randomization codes. The actual allocation will only be provided to the study team after locking of the database and approval of the statistical analysis plan.

Trial drugs

After randomization, the participant will receive:

1. Thymosin Alpha 1 1.6mg I.H q12h for the first seven days and 1.6mg I.H, qd for the following seven days. The administration will be terminated any day during the treatment when the patient is deemed as qualified for hospital discharge, or dead.
2. Matching placebo(normal saline) using the same mode of administration as the above mentioned.

As shown in Figure 1, the recruited patients will start to receive randomized drugs subcutaneously from the day after the allocation day. Thymosin Alpha will be provided by SciClone Pharmaceuticals and the matching placebo by Chengdu Tongde Pharmaceuticals. All study drugs will be stored in a secure area with access limited to the investigators and authorized study site personnel, and under appropriate storage conditions.

General treatment regimen

All patients will receive standard treatment including fluid therapy, early enteral nutrition, routine medical treatment like proton pump inhibitor as indicated, mechanical

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ventilation if needed, and continuous renal replacement therapy (CRRT) if needed in the light of recently published guidelines[17]. All participating centers are able to offer appropriate intensive care in case the patients require organ support or continuous monitoring. The necrotic collection will be intervened when infection is suspected or confirmed, preferably after four weeks from the onset of the disease when the patient could tolerate the symptoms as suggested by the guidelines[17].

When pancreatic infection occurs, either a surgical or endoscopic step-up approach considering the location of the necrotic collection and the technical availability in each participating center will be applied. Principally, either percutaneous catheter drainage or endoscopic transluminal placement of double pig-tail stents， rather than debridement, are the primary choices of treatment.

Endpoints

Primary outcome measure

The incidence of IPN during the index admission will be served as the primary outcome measure of the TRACE trial. The diagnosis of IPN will be based on the international guidelines when one or more of the following were present: gas bubbles within (peri) pancreatic necrosis on computed tomography; a positive culture of (peri) pancreatic necrosis obtained by image-guided fine-needle aspiration; a positive culture of (peri) pancreatic necrosis obtained during the first drainage and/or necrosectomy[15].

Secondary outcome measures

Part I：Secondary outcomes during the index admission

1. The occurrence of new-onset organ failure and new-onset persistent organ failure (SOFA score for respiration, cardiovascular, or renal system ≥2). New-onset is defined as events that occur after randomization and not present 24 hours before randomization;
2. In-hospital mortality;
3. Bleeding requiring intervention；
4. Gastrointestinal perforation or fistula requiring intervention;
5. Incidence of pancreatic fistula

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6. New receipt of mechanical ventilation (not applied 24 hours before randomization);
7. New receipt of renal replacement therapy (not applied 24 hours before randomization);
8. New receipt of vasoactive agents (not applied 24 hours before randomization);
9. The requirement for catheter drainage (either percutaneous or endoscopic)
10. Number of drainage procedures required;
11. The requirement for minimally-invasive debridement;
12. Number of minimally invasive necrosectomy required;
13. The requirement for open surgery;
14. Number of open operations required;
15. Length of intensive care unit(ICU) stay;
16. Length of hospital stay;
17. SOFA score on day0, day7, and day14；
18. CRP level on day0, day7, and day14;
19. HLA-DR level on day0, day7, and day14;
20. Lymphocyte count on day0, day7 and day 14;
21. In-hospital cost.

Part II：Secondary outcomes within 90 days after enrollment

1. Incidence of infection within 90 days after enrollment；
2. Mortality within 90 days after enrollment.

Sample size estimation

The incidence of IPN during the index admission was reported to be around 25% in ANP episodes combined with an APACHE II score≥8 in our previous studies[18, 19]. To reduce the incidence of IPN from 25% to 15% on the basis of our pilot study [14], we projected a sample size of 500 participants with 80% power at a two-sided alpha level of 0.05 using the PASS software (PASS 11, NCSS software, Kaysville, USA). In our study, we planned to randomize 520 patients after considering 4% of lost follow up.

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Statistical analysis

Primary analyses will be based on the intention-to-treat (ITT) population, and secondary supportive analyses will be done on the PP population. The safety analysis will be performed on the safety population. Missing data will be handled by multiple imputations to evaluate the robustness of the primary endpoint analyses[20]. The populations are defined as follows:

1. ITT population: This population consists of all randomized subjects, regardless of whether they are ineligible, prematurely discontinue treatment, or are otherwise protocol violators/deviators.
2. Per-protocol (PP) population: This population is a subset of the ITT population. Subjects with major protocol deviations will be excluded from the PP population. Major protocol deviations will be defined in the statistical analysis plan.
3. Safety population: This population will be the same as the ITT population, which consists of all randomized subjects, who receive at least one dose of study drug.

The normality of continuous variables was examined using skewness and kurtosis. Categorical data were expressed as number and percentage. A generalized linear model (GLM) will be employed to compare group differences in the primary outcome. No interim analysis was planned in our study. The detailed analysis strategies for secondary outcomes and subgroup analyses by the severity of AP(severe and non-severe), age(dichotomized at 60 years old), etiologies of AP (biliary and non-biliary) and extent of pancreatic necrosis(>50% and ≤50%), will be included in the statistical analysis plan. Statistical tests will be two-sided, and p values < 0.05 will be deemed as significant.

Adverse events

Adverse events (AEs) are defined in accordance with the National Cancer Institute-Common Terminology Criteria for Adverse Events as any untoward medical occurrence in a patient, or clinical investigation subject administered an investigational intervention and which does not necessarily have to have a causal relationship with this

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treatment.

It is recognized that the study patient population (ANP with relatively high APACHE II score) will experience a number of common aberrations in laboratory values, signs, and symptoms due to the severity of the underlying disease and the impact of standard therapies. These will not necessarily constitute an adverse event unless they require significant intervention or are considered to be of concern in the investigator's clinical judgment. Thymosin alpha 1 is a well-studied drug with a favorable safety profile in previous trials [21]. The DSMB will review the safety report every six months.

Recruiting process

The trial was registered on June 16th, 2015, in the CT.gov registry(NCT02473406 https://www.clinicaltrials.gov). The first patient was randomized on March 22nd, 2017. So far, 426 patients had been randomized, and the enrollment keeps to the schedule.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Data collection and management

A web-based electrical database (access through the website of the CAPCTG, https://capctg.medbit.cn/) will be used for data collection and storage. All data will be input by the primary investigator or nominated investigators (less than two for each participating center) approved by the primary investigator, and a double check will be done by the research coordinator. Training for data entry will be performed by the provider of the electrical database (Unimed Scientific Inc., Wuxi, China) and the coordinating and data management center of the CAPCTG. According to the schedule shown in Figure 2, the investigator will collect data during the index admission and on day 90 after enrollment. If a study subject wishes to discontinue the study drug or the treating physician believes a study subject should discontinue the study drug due to

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medical considerations, the investigator will communicate with the study subject and the treating physician to obtain the reasons. Further evaluation and follow-up will still be performed unless the study subject withdraws consent for disclosure of information. The study blinding will only be broken in a medical emergency when the treating physician believes that the administration of the study drug is associated with the emergency.

Ethics approval and dissemination

This study was approved by the ethics committee of Jinling Hospital. The ethical approval document ID is 2015NZKY-004-02. Even when central ethical approval has been confirmed, we will not begin recruiting at other participating centers in the trial until the local ethics committee approved the study. Site ethical approvals were obtained from ethics committees of First Affiliated Hospital of Nanchang University, The Affiliated Hospital of Qingdao University, Affiliated Hospital of Zunyi Medical College, Nanhua Hospital, Second Affiliated Hospital of Nantong University, Yijishan Hospital of Wannan Medical College, 908th Hospital of Chinese People's Liberation Army, Jiangsu Province Hospital on Integration of Chinese and Western Medicine, Zhejiang Provincial People's Hospital, Luoyang Central Hospital, The Affiliated Hospital of Henan University of Science and Technology, Northern Jiangsu People's Hospital, First People's Hospital of Shangqiu, Qilu Hospital of Shandong University, and First Affiliated Hospital of Anhui Medical University. The results of this trial will be reported in peer-reviewed journals and presented at scientific conferences.

Consent to participate

The consents for this study is obtained from each patient or his/her next of kin with full information regarding the possible adverse effects of the experimental drug and potential consequences. The translated patient consent form is attached as a supplemental file(Supplement Materials).

Discussion

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The TRACE trial was designed to test the efficacy of a new therapy targeting the immune system in preventing IPN following ANP, which is a potentially lethal complication causing substantial morbidity and mortality. We also aimed to investigate the efficacy of immunomodulatory treatment with thymosin alpha 1 in patients with different clinical characteristics using predefined subgroup analysis. The results of the TRACE trial would potentially provide a novel therapeutic option in the management of ANP and identify the patient population who may benefit most from the administration of thymosin alpha 1.

Immunomodulation is of significant clinical value in critically ill settings and the treatment of sepsis[12]. While, acute pancreatitis, which has a lot in common with sepsis like overwhelmed inflammation and infection related complications, might be another suitable target for immunomodulatory therapy. In general, previously studied drugs such as lexipafant and octreotide were aimed to control cytokines, which are thought to be the pivotal part in the early inflammatory response of AP, rather than preventing the development of IPN[11]. However, like what we learned in sepsis, immunosuppression quickly following the initial inflammatory cascades should be the target of treatment during the course of ANP, as well, especially in those with organ failure[8, 22]. A pilot study published by our group several years ago indicated that the administration of thymosin alpha 1 could improve compromised monocyte HLA-DR expression and reduce infection rate in a small group of patients (n=24) with severe acute pancreatitis defined by the original Atlanta Classification. The result of this study is encouraging which drive us to conduct this large multi-center RCT to obtain more reliable clinical evidences[14].

The TRACE trial was sponsored by the CSAP at Jinling Hospital, Nanjing University, which is the national referral center for acute pancreatitis (AP) admitting more than 600 cases of AP annually and coordinated by the CAPCTG coordinating and data management center, which could cover the whole country. The trial is performed in 16 centers across China and aims to recruit 520 patients. Due to the limitation of the budget and technical availability, we can not conduct a continuous immune assessment with multiple markers and more time points. Alternatively, we choose monocyte HLA-DR,

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which is a representative parameter of the immune system, majorly reflecting the antigen presentation capacity to assess the immunomodulatory effect of thymosin alpha 1. HLA-DR was widely used in previous studies regarding immune function in different diseases like sepsis[12, 23].

In conclusion, the TRACE trial aims to assess the efficacy of thymosin α1 administered early during the ANP on the incidence of IPN and other major clinical outcomes and thereby potentially offer a novel therapeutic option in the treatment of ANP patients.

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Figure 1：Trial flow chart.

Figure 2. Schedule for participants enrolment, drug administration, and data collection.

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Declarations

Authors' contributions：

All authors were involved in the study design, and read and approved the final manuscript. During the study, J Z, W M and Y L are responsible for randomizing the patients and ensuring the blinding. J Z, W H, X P，M C，C H, W G, J W, J S, H N, J T, J S，G Z, W C, B X, X Z, M S are responsible for carrying out recruitment, managing the treatment of the patients and collecting data. W L,Z T,L K,J Z,W M and W H, X P，M C，C H, W G, J W, J S, H N, J T, J S，G Z, W C, B X, X Z, M S are members of the trial steering committee. J Z , L K, Z T and T C drafted the manuscript.

Competing interests

This study is supported by SBE2016750187 of Science and technology project, Jiangsu Province, China. SciClone Pharmaceuticals provides the study drug for this investigator-initiated study but has no influence on the study design, data analysis, or report. The investigators take full responsibility for the integrity and content of this paper.

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This study is funded by SBE2016750187 of Science and technology project, Jiangsu Province, China, partly by SciClone Pharmaceuticals Holding Limited. The sponsor had no role in study design, data collection, and interpretation, or in the decision to submit the manuscript for publication.

Consent for publication

Not Applicable.

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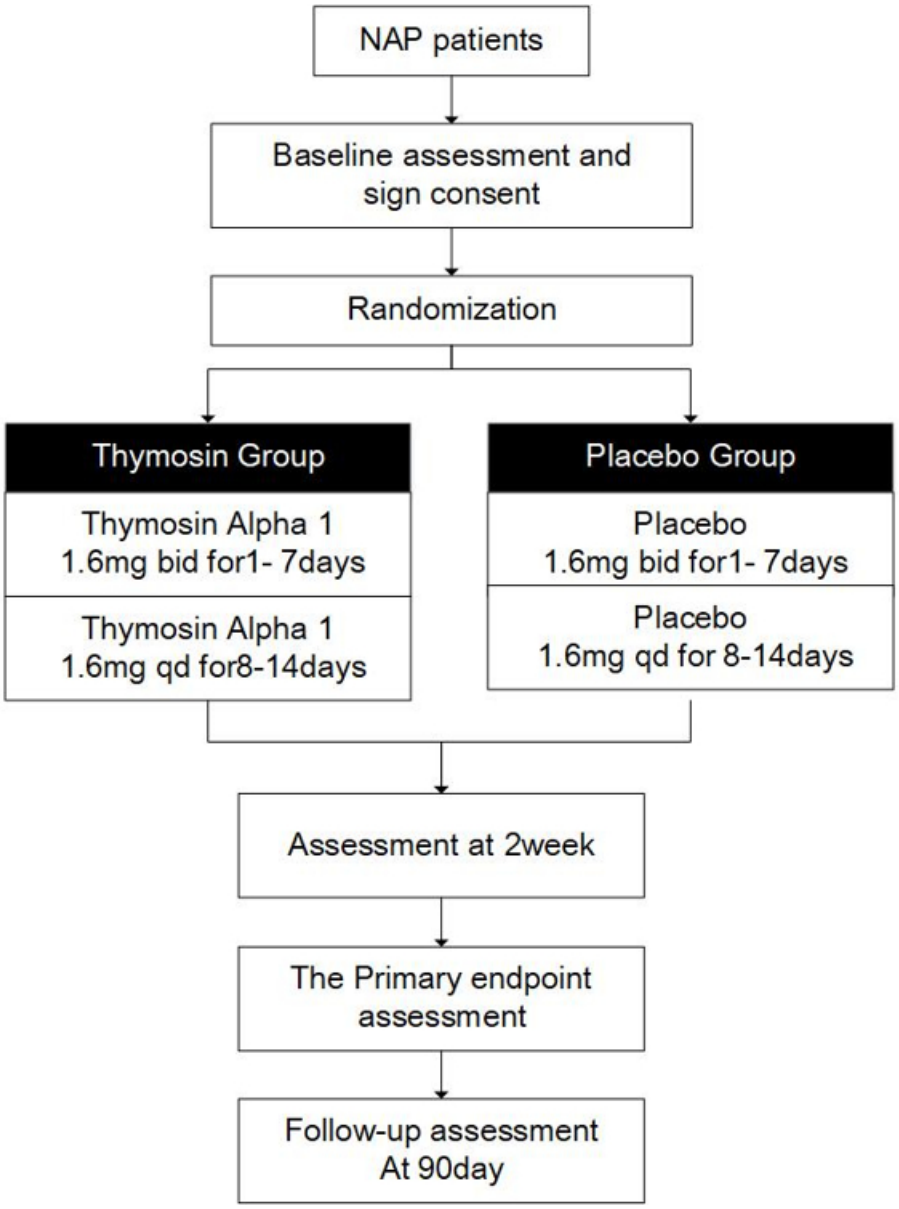

Figure 1 : Trial flow chart.

	Study period							
	Enrollment	Allocation	Index admission					Follow-up
TIMEPOINT	< 24h	0	day1-6	day7	day8-13	day14	discharge/death	90 day
ENROLLMENT								
Eligibility screening	X							
Informed consent	X							
Allocation		X						
INTERVENTIONS:								
Drug injection 1.6mg bid			X	X				
Drug injection 1.6mg qd					X	X		
ASSESSMENTS:								
Incidence of IPN			←	—	—	→		
Major complications			←	—	—	→		
Laboratory test	X			X		X		
Organ failure assessment	X			X		X		
Status of vitality and infection								X

Figure 2. Schedule for participants enrolment, drug administration, and data collection.

Jinling Hospital, Nanjing University

CONSENT FORM

TITLE OF STUDY: Thymosin alpha 1 in the prevention of infected pancreatic necrosis following acute necrotizing pancreatitis

PRINCIPAL INVESTIGATOR: Weiqin Li, M.D.

We are conducting a clinical trial (a type of research study). Clinical trials include only patients who choose to take part in the study. This consent form serves two purposes. First, it provides information on the procedures and risks involved in the clinical trial, so that you can decide if you want to take part in the study.

Second, this form will ask for your permission to use and release the medical information that we will get from you during this study. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. If you have any questions, you can ask the study doctor for more explanation.

This study is being sponsored by Jinling Hospital of Nanjing University. You are being asked to take part in this study because you were admitted to Jinling Hospital with acute necrotizing pancreatitis (ANP).

WHY IS THIS STUDY BEING DONE?

Infected pancreatic necrosis (IPN) and its related septic complications are the major causes of death in patients with ANP. Our previous study found that Thymosin alpha 1 is associated with improved cellular immunity and reduced infection rate in a group of ANP patients. However, no further study was published yet, and the clinical significance of this study is limited due to the small sample size and single-center nature. Therefore, we conduct a multicenter, randomized study with a sufficiently-powered sample size and proper design to evaluate the efficacy and safety of Thymosin Alpha 1 in treating ANP.

It is planned that a total of 520 people will take part in this study from 10-20 hospitals within China.

WHAT WILL HAPPEN IN THE STUDY?

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You will be "randomized" into one of the study groups described below. Randomization means that you are put into a group by chance. It is like flipping a coin. Neither you nor the study doctor will choose what group you will be in. You will have an equal chance of being placed in either group.

Group 1 will receive Thymosin Alpha 1(ZADAXIN) 1.6mg I.H q12h for the first seven days and 1.6mg I.H, qd for the following seven days. Group 2 will receive a matching placebo (normal saline) using the same mode of administration, as mentioned above. The administration will be terminated any day during the treatment when the patient deemed as qualified for discharge

You will be in the study until you are discharged from the hospital.However,You can stop being a part of this study at any time. Iif you decide to stop being in the study, please talk to the study doctor first.

WHAT ARE THE RISKS OF THE STUDY?

While on the study, you are at risk for the following side effects. You should discuss these with the study doctor. We will be looking at your medical records up to 90 days after "randomization." If you are discharged before that, you will be contacted by phone.

Risks and side effects related to the study drug(Thymosin Alpha 1) and placebo(normal saline) include:

- primarily local discomfort at the injection site
- rare instances of erythema
- transient muscle atrophy
- polyarthralgia combined with hand edema,
- skin rash

There also may be other side effects that we cannot predict. These side effects are often manageable and reversible. You will be observed for side effects, and all medically appropriate efforts will be made to prevent and/or control them. If there are side effects that cannot be controlled or reversed, they may result in serious injury or death.You should report any of these problems to the study doctor immediately so that appropriate care can be given.Side effects other than those listed here may also occur.Talk to the study doctors about any side effects that seems unsual or that is especially bothersome to you.

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ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

If you agree to take part in this study, you are free to use the Thymosin Alpha 1(ZADAXIN)/placebo provided by Sciclone Pharmaceuticals (CHINA). Besides, you will experience improved access to medical care, more efficient medical evaluation, and management. The results of this study could help future patients with your condition.

WHAT ARE THE COSTS?

Taking part in this study will not lead to added costs to you or your insurance company. The trial drug and related immune system laboratory tests will be provided free of charge. The sponsor will not pay for routine costs required during hospitalization.

While you are in this study, you may receive tests, procedures, and exams that are standard medical care. This standard medical care may or may not be covered by your medical insurance.If your medical insurance does not pay for this standard medical care, you will be responsible for the cost of medical care related to your condition.

COMPENSATION?

You will receive no payment for taking part in this study.

WHAT ABOUT CONFIDENTIALITY?

Only the medical information that will be collected from you if you take part in this study.Date masking will be performed to protect your Identification information from divulging.The medical information are as follows:

- Information obtained from procedures used to determine your eligibility to take part in the study, including a routine medical history, physical exam, and laboratory data.
- Information that is created or collected from you during your participation in the study, including your overall medical condition and follow-up information up to 90 days after randomization.
- Information contained in your underlying medical records related to your medical history and treatment prior to this study

Only the hospitals envolved in the study may inspect and/or copy your research records for quality assurance and data analysis.

DO I HAVE THE RIGHT TO DECLINE AUTHORIZATION?

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You have the right to decline to sign this authorization to use/disclose your medical information. If you decline, you will not be able to take part in this research study. Except as described herein, if you decline to sign this authorization, your rights concerning treatment, payment for services, enrollment in a health plan, or eligibility for benefits will not be affected.The authorization for use and disclosure of your information will remain in effect until the study is complete.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Your doctor has answered your questions. You can ask your doctor questions at any time. Taking part in this study is voluntary. You may choose not to take part, or you may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. We will tell you about the important new information that may affect your health, welfare, and willingness to stay in this study.

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STATEMENT OF CONSENT AND AUTHORIZATION

I hereby freely and voluntarily consent to take part in the research study described above. This consent is given based on the verbal and written information provided and the understanding that I am medically and physically qualified to take part in this study. I am free to ask questions at any time.

I have the option to decline to take part or to withdraw from the study at any time without incurring any penalty or loss of benefits otherwise available, including medical care at this institution.

My signature below indicates that I voluntarily agree to take part in this study and that I authorize the use and disclosure of my information in connection with the study. I will receive a signed copy of this consent and authorization form.

Patient Signature*	Date	Time
Research Coordinator Signature	Date	Time
Investigator Signature	Date	Time

*If this consent is signed by a legal representative of the patient, check applicable box below explaining your authority to sign for the patient. For legal representatives acting in the capacity as a parent/guardian to the patient, attach a copy of documentation giving you the authority to sign this consent form on behalf of the patient.

- o Next of Kin
- o Parent (patient is a minor)
- o Guardian
- o Other relationship: _____

Signature of Patient's Legally Authorized Representative	Date	Time
Witness to consent process(if applicable)	Date	Time

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	page/line numbers
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P1/L1-4
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P3/L76-77
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	P17/L481-484
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P1/L5-46
	5b	Name and contact information for the trial sponsor	P2/L47-51
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P17/L469-474
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	P6/L141-147
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P5/L92-127
	6b	Explanation for choice of comparators	P5/L92-127

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Objectives	7	Specific objectives or hypotheses	P6/L130-133
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P6/L136-137 P8/L182-184
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P6/L138-140
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P7/L154-179
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P8/L194-206
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P12/L327-331
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P10/L255-256
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P8/L209-221
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P9/L224-261
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P16/L452
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P10/L264-268
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P12/L310-312
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

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Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P8/L184-185
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	P8/L187-190
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P8/L184-191
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P8/L186-189
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	P12/L332-334
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P12/L319-327
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P12/L327-331
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P12/L319-326
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P11/L272-287
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P11/L288-293

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	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P11/L277-284
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P6/L145-147
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	P11/L288
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P11/L296-305
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P12/L307
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P13/L337-350
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	Not applicable
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P13/L353-356
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Detail in informed consent form
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Detail in informed consent form
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P17/L476-479

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Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Detail in informed consent form
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Detail in informed consent form
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P12/L349-350
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	See supplement materials
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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